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The Role of New Posaconazole Formulations in the Treatment of *Candida albicans* Infections: Data from an *In Vitro* Pharmacokinetic-Pharmacodynamic Model

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ABSTRACT Posaconazole is more active than fluconazole against *Candida albicans* *in vitro* and is approved for the treatment of oropharyngeal candidiasis but not for that of invasive candidiasis (IC). Here, we explored the efficacy of posaconazole against *C. albicans* in an *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) model of IC and determined the probability of pharmacodynamic target attainment for the oral solution and intravenous (i.v.)/tablet formulations. Three clinical *C. albicans* isolates (posaconazole MICs, 0.008 to 0.25 mg/liter) were studied in the *in vitro* PK/PD dilution model simulating steady-state posaconazole PK. The *in vitro* exposure-effect relationship, area under the 24-h free drug concentration curve ($fAUC_{0-24}$)/MIC, was described and compared with *in vivo* outcome in animals with IC. PK/PD susceptibility breakpoints and trough levels required for optimal treatment were determined for EUCAST and CLSI 24-h/48-h (CLSI24h/CLSI48h) methods using the $fAUC_{0-24}$/MIC associated with half-maximal activity (EI_{50}) and Monte Carlo simulation analysis for oral solution (400 mg every 12 hours [q12h]) and i.v./tablet formulations (300 mg q24h). The *in vitro* mean (95% confidence interval [CI]) EI_{50} was 330 (183 to 597) $fAUC_{0-24}$/MIC for CLSI24h and 169 (92 to 310) for EUCAST/CLSI48h methods, which are close to the near-stasis *in vivo* effect. The probability of target attainment for EI_{50} was estimated; for the wild-type isolates (MIC $\leq$ 0.06 mg/liter), it was low for the oral solution and higher than 95% for the i.v./tablet formulations for the EUCAST/CLSI48h methods but not for the CLSI 24-h method. Non-wild-type isolates with EUCAST/CLSI48h MICs of 0.125 and 0.25 mg/liter would require trough levels of $>$1.2 and $>$2.4 mg/liter, respectively. Posaconazole i.v./tablet formulations may have a role in the therapy of invasive infections by wild-type *C. albicans* isolates, provided that a steady state is reached quickly. A PK/PD susceptibility breakpoint at the epidemiological cutoff (ECV/ECOFF) of 0.06 mg/liter was determined.

KEYWORDS posaconazole, *Candida albicans*, PK/PD susceptibility breakpoints, CLSI, EUCAST, Monte Carlo simulation, breakpoints, candidiasis, pharmacokinetic-pharmacodynamic, therapeutic drug monitoring

Posaconazole is a triazole antifungal agent. The licensed indications include first-line therapy for the treatment of oropharyngeal candidiasis and prophylaxis of invasive fungal infections, including those caused by *Candida*, but not for other *Candida* infections (1). Generally, posaconazole exhibits equal or superior *in vitro* activity to those of fluconazole and voriconazole against *Candida albicans* isolates (2, 3). Notably,

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TABLE 1 *In vitro* susceptibility testing of *C. albicans* isolates with EUCAST and CLSI reference methods tested in triplicate

C. albicans isolate no.	Reference code	Median MIC (range) in mg/liter determined by:		
		EUCAST	CLSI24h	CLSI48h
1	2-76	0.016 (0.016–0.03)	0.008 (0.008)	0.016 (0.016)
2	K1	0.03 (0.016–0.06)	0.016 (0.008–0.016)	0.03 (0.008–0.03)
3	98-210	0.25 (0.125–0.5)	0.125 (0.06–0.125)	0.25 (0.125–0.5)

differential activity has been demonstrated for some isolates with target gene mutations similar to what is found for azoles and *Aspergillus fumigatus*. Thus, either some *ERG11* mutations have no effect on posaconazole MICs while increasing fluconazole and voriconazole MICs or multiple mutations in the *ERG11* gene are required to confer decreased posaconazole susceptibility (4–6). In addition, the newly approved formulations of posaconazole (delayed-release oral tablet and intravenous formulation) (7, 8), compensating for the restricted bioavailability of the oral solution of posaconazole and suboptimal drug exposure, represent a new option for the prophylaxis and treatment of invasive fungal infections.

In the present study, we investigated the pharmacodynamics of posaconazole against *C. albicans* isolates with an *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) dilution model simulating posaconazole pharmacokinetics. After validating the model against the results obtained from an animal model of experimental invasive candidiasis using the same *C. albicans* strains (9), the *in vitro* exposure-effect relationship was described and the probability of pharmacodynamic target attainment was estimated for the oral solution and the new intravenous (i.v.) and tablet formulations. Finally, PK/PD susceptibility breakpoints and target serum values for therapeutic drug monitoring was determined for the current CLSI and EUCAST reference methods.

RESULTS

Susceptibility testing. Posaconazole MICs for each isolate determined by EUCAST and CLSI methods after 24 and 48 h are shown in Table 1. MICs among methods were within ±1 2-fold dilutions, with the best absolute agreement found between the EUCAST and CLSI 48-h (CLSI48h) method MICs, since all CLSI 24-h (CLSI24h) MICs were 1 2-fold dilution lower than EUCAST MICs.

Simulation of the mouse model. (i) Pharmacokinetics. Posaconazole time-concentration profiles in the *in vitro* model were characterized by maximum concentration of free unbound fraction (fC_{max}; mean ± standard deviation [SD] of all experiments for the first and second 24 h) values of 0.25 ± 0.04, 0.36 ± 0.06, 4.92 ± 0.94, and 6.92 ± 2.13 mg/liter and $f\text{AUC}_{0-24}$ values of 1.56 ± 0.30, 2.94 ± 1.19, 44.33 ± 4.69, and 80.82 ± 15.62 mg · h/liter, respectively, with a mean half-life $t_{1/2}$ (range) of 8 (4 to 13) h (Fig. 1). The corresponding values for the lower dose, with a fC_{max} of 0.15 mg/liter, were extrapolated, since concentrations were below the detection limit of the bioassay.

(ii) Pharmacodynamics. Fungal load increased from a mean ± SD (among isolates) of 3.61 ± 0.07 $\log_{10}$CFU/ml at $t = 0$ h to 6.69 ± 0.36 $\log_{10}$ CFU/ml at $t = 48$ h in drug-free controls, whereas a 1 to 4 $\log_{10}$CFU/ml reduction of fungal load compared to that in drug-free control was observed at different posaconazole exposures after 48 h, in line with *in vivo* findings (9) (Fig. 2). A maximum effect corresponding to a 1 to 2 $\log_{10}$ kill compared to initial inoculum was found at high posaconazole exposures, as also observed in animal experiments (9). When dilution of CFU during the experiment was taken into account and $\log_{10}$CFU was analyzed, time-kill curves were shifted upwards by <0.5 $\log_{10}$CFU because of a maximal 4× increase of the volume of the central compartment at 48 h (see Fig. S1 in the supplemental material). The *in vitro* exposure-effect relationship for the *C. albicans* isolates followed a sigmoid curve with a mean (95% confidence interval [CI]) half-maximal activity (EI_{50}) of 83 (46 to 146) after 24 h and 169 (92 to 310) after 48 h using the CLSI48h MICs (R^2 = 0.85 to 0.87) (Fig. 3). The *in vitro*

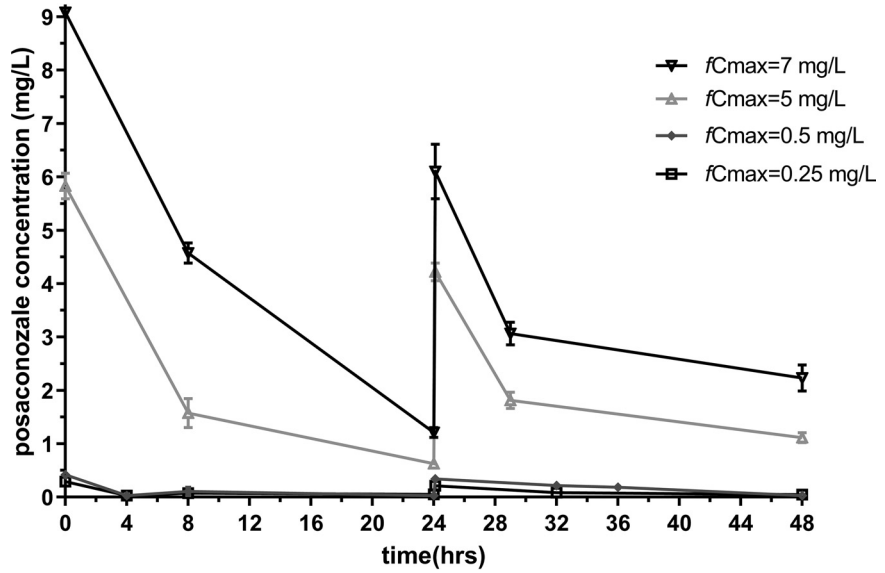

FIG 1 Representative time-concentration profiles of simulated every 24 hours (q24h) dosing regimens of posaconazole in the *in vitro* PK/PD model with target fC_{max} values of 0.25, 0.5, 5, and 7 mg/liter, respectively, and a half-life ($t_{1/2}$) of 8 (4 to 13) h. Error bars represent standard deviation (SD).

exposure-effect relationship for the three *C. albicans* isolates followed a sigmoid curve (R^2 = 0.85 to 0.88) with mean (95% CI) 48-h EI_{50} values of 169 (92 to 310) for EUCAST and 330 (182 to 597) $fAUC_{0-24}$/MIC for CLSI24h. The 48-h EI_{50} was approximately two times higher than the 24-h EI_{50} (Fig. 4). Similar PK/PD targets were found when

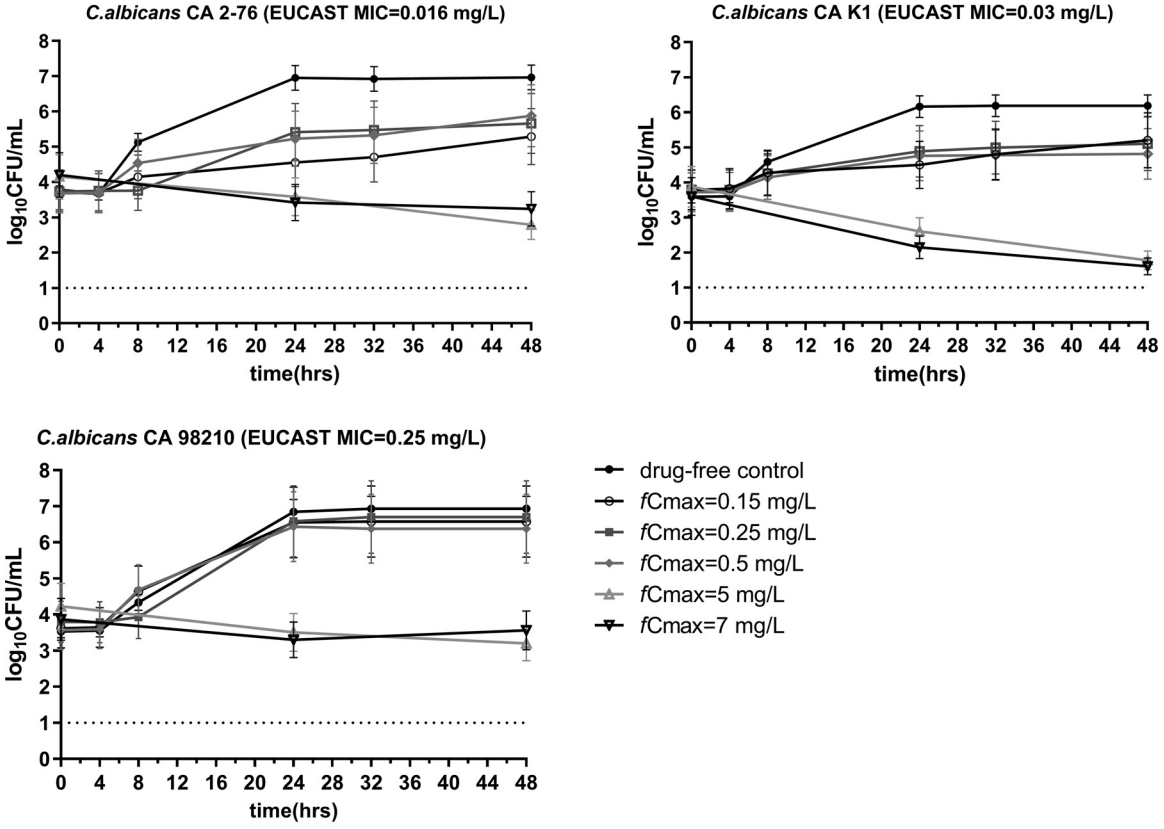

FIG 2 Time-kill curves in the *in vitro* PK/PD model simulating animal q24h oral dosing regimens of posaconazole against *C. albicans* targeting different fC_{max} values with a $t_{1/2}$ of 8 (4 to 13) h. Error bars represent SD.

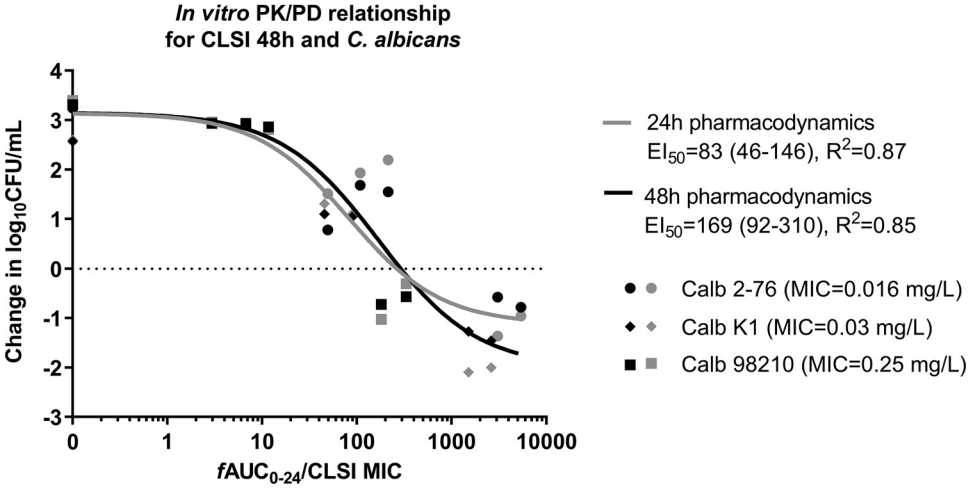

FIG 3 *In vitro* PK/PD relationship of posaconazole against *C. albicans* as a function of 24-h and 48-h changes in $\log_{10}$CFU/ml from initial inoculum and $f\text{AUC}_{0-24}$/MIC ratios of different simulated animal PK profiles based on CLSI48h MICs.

$\log_{10}$CFU was analyzed taking into account the dilution of CFU (see Fig. S2 and S3 in the supplemental material).

Posaconazole pharmacodynamics in human serum. Time-kill curves in 100% human serum and in RPMI 1640 medium are shown in Fig. 5. Although the fungal burden in drug-free controls with and without serum were different, the $\log_{10}$CFU/ml value at a total C_{max} (tC_{max}) of 7 mg/liter in human serum was similar to that at an fC_{max} of 7 mg/liter in RPMI medium, close to the initial inoculum, indicating that serum does not affect static exposure of posaconazole.

PK/PD target attainment. For the 400-mg every 12 hours (q12h) oral solution of posaconazole, the mean (95% confidence interval) probabilities of target attainment (PTA) for the EUCAST/CLSI48h PK/PD target of 169 (92 to 310) $f\text{AUC}_{0-24}$/MIC were 89% (66 to 98%), 62% (30 to 86%), and 26% (7 to 57) for EUCAST/CLSI48h MICs of 0.03, 0.06, and 0.125 mg/liter, respectively (Fig. 6). In a similar analysis for the CLSI24h PK/PD target of 330 (183 to 597) $f\text{AUC}_{0-24}$/MIC, the mean (95% confidence interval) PTA were 91% (70 to 98%), 63% (32 to 86%), and 27% (8 to 58%) for CLSI24h MICs of 0.016, 0.03, and 0.06 mg/liter, respectively. For the 300-mg q24h tablet/i.v. formulations of posaconazole and the EUCAST/CLSI48h methods, the PTA were higher than 95% for MICs of $\leq$0.06 mg/liter and significantly higher than 20% (i.e., lower 95% CI limit of PTA higher than 20%) for MICs of 0.125 to 0.25. For the CLSI24h method, the PTA were higher than 85% (i.e., lower 95% CI limit of PTA higher than 85%) for MICs of $\leq$0.03 mg/liter and higher than 20% for MICs of 0.06 to 0.125 mg/liter. For higher MICs, the PTA were 0% (Fig. 6). Because the tablet and i.v. formulations of posaconazole result in similar AUC_{0-24} values, the PTA were similar (data not shown).

Therapeutic drug monitoring. The steady-state trough levels and total AUC_{0-24} ($t\text{AUC}_{0-24}$) required to attain the PK/PD targets for the three dosing regimens were calculated for different isolates with increasing CLSI24h and EUCAST/CLSI48h MICs (Fig. 7). The AUC/MIC and trough/MIC ratios of 165 and 12.69 for 400 mg q12 oral solution, 330 and 10.31 for the i.v./tablet dosing regimens for CLSI24h, 84.5 and 6.5 for 400 mg q12h oral solution, and 169 and 5.28 for the i.v./tablet dosing regimens for EUCAST/CLSI48h were used to correlate drug exposure in patients with different MICs of *C. albicans* isolates. Isolates with CLSI24h and EUCAST/CLSI48h MICs of $>$0.125 mg/liter and $>$0.25 mg/liter, respectively, would require clinically unachievable concentrations with either formulation, verifying the 0% PTA found above. For the oral solution, the PK/PD target could be safely attained for isolates with a CLSI24h MIC of 0.06 mg/liter and EUCAST/CLSI48h MICs of 0.125 mg/liter with stable trough levels of 0.8 mg/liter (upper 95% CI limit, 1.5 mg/liter). For the i.v./tablet formulations, the PK/PD target could be

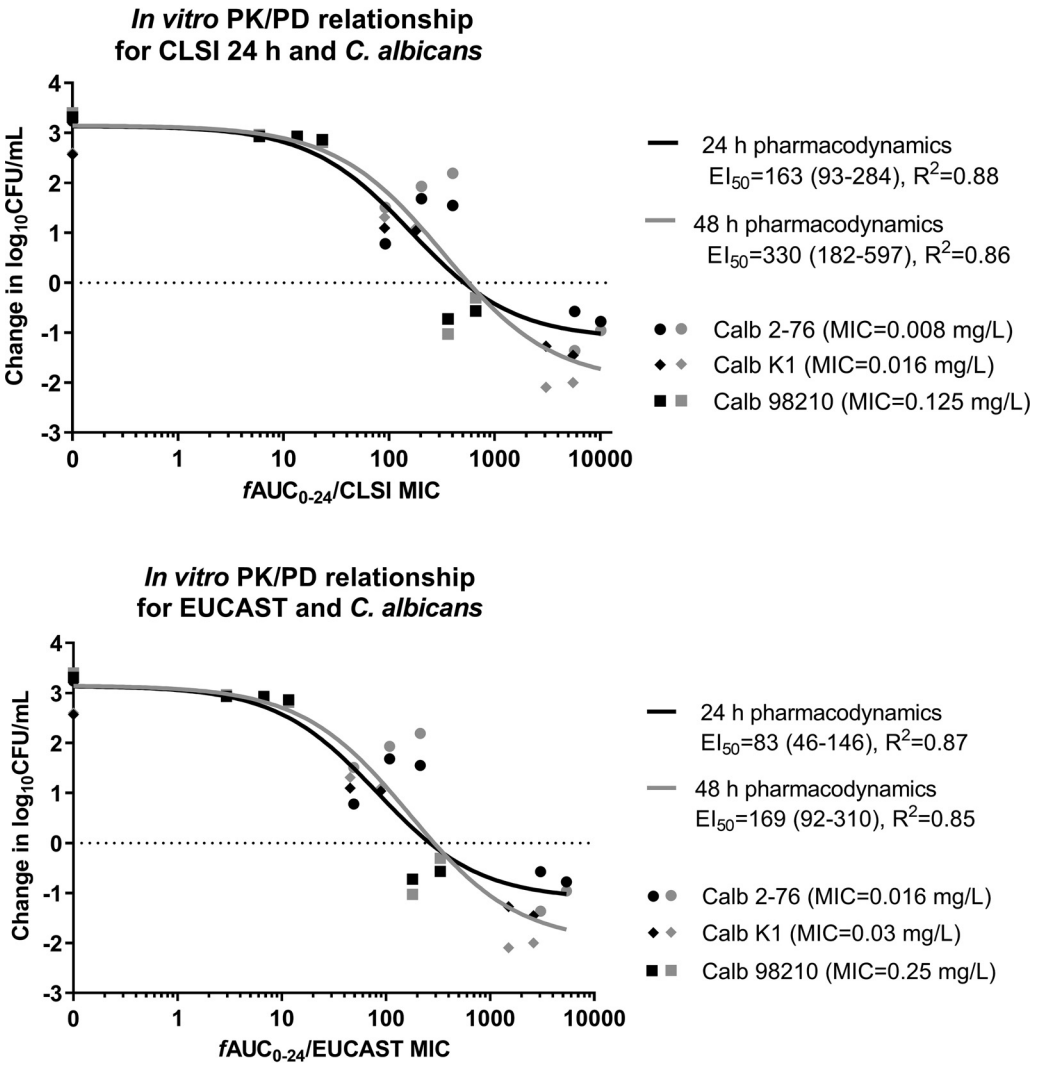

FIG 4 *In vitro* PK/PD relationship of posaconazole against *C. albicans* as a function of 24-h and 48-h changes in log_{10}CFU/ml from initial inoculums and $fAUC_{0-24}$/MIC ratios of different simulated animal PK profiles based on EUCAST and CLSI24h MICs.

attained for isolates with CLSI24h MICs of 0.125 mg/liter and EUCAST/CLSI48h MICs of 0.25 mg/liter with stable trough levels of 1.3 mg/liter (upper 95% CI limit, 2.4 mg/liter).

DISCUSSION

An *in vitro* PK/PD model was used to determine PK/PD breakpoints for posaconazole against *C. albicans* for EUCAST and CLSI reference methods. The *in vitro* model was validated using the same *C. albicans* isolates previously used in an animal neutropenic *in vivo* model of disseminated candidiasis. Animal serum posaconazole pharmacokinetics were simulated in order to describe the *in vitro* PK/PD relationship, resulting in mean (95% CI) 48-h PK/PD indices of 169 (92 to 310) for EUCAST/CLSI48h methods and 330 (183 to 597) $fAUC_{0-24}$/MIC for the CLSI24h method. The 24-h PK/PD targets were two times lower than the 48-h PK/PD targets, as previously found for voriconazole against *Candida* spp. (10, 11), indicating that at least 48 h are required in order to describe the pharmacodynamics against *Candida* spp. Based on 48-h PK/PD indices, the oral solution would not cover the wild-type population of *C. albicans* ($\leq$0.06 mg/liter) without therapeutic-drug monitoring (TDM) targeting trough (upper 95% CI limit) levels of $>$0.7 mg/liter for EUCAST/CLSI48h and $>$1.4 mg/liter for CLSI24h. For the i.v./

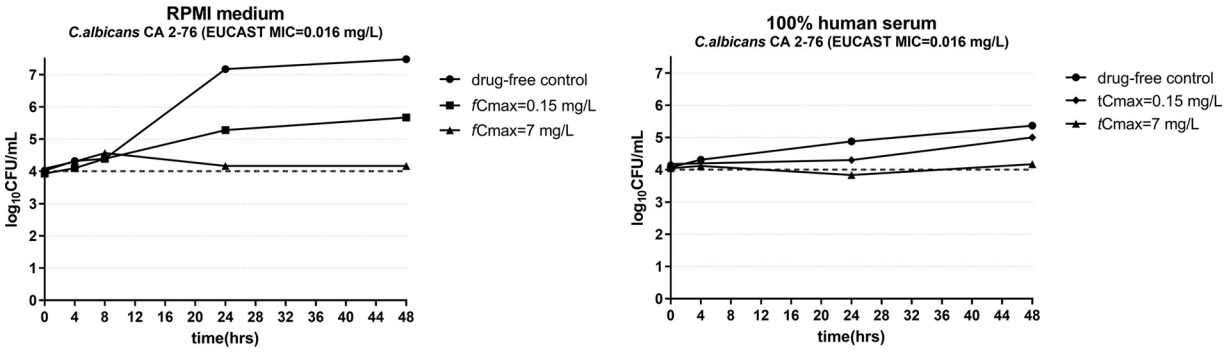

FIG 5 Comparative *in vitro* pharmacodynamics of posaconazole in RPMI medium and in 100% serum. Time-kill curves of a C_{max} of 7 mg/liter of posaconazole in the presence and absence of 100% human serum, as well as the corresponding fC_{max} of 0.15 mg/liter of posaconazole in serum-free medium (protein binding, 98%) against *C. albicans* isolate 2-76. Note that stasis (horizontal broken line) was found with 7 mg/liter of posaconazole with and without serum.

tablet formulations, the PTA for the wild-type population were >95% for EUCAST/CLSI48h, while for CLSI24h the PTA were lower (PTA, 86% [35 to 100%]). The PK/PD targets for EUCAST/CLSI48h and CLSI24h non-wild-type isolates with MICs of 0.125 mg/liter could be attained with trough (upper 95% CI limit) levels of >1.2 and >2.4 mg/liter, respectively, for the i.v./tablet formulation. Because of the 2-fold lower EUCAST/CLSI48h MICs and PK/PD targets compared to those for CLSI24h, non-wild-type isolates with an EUCAST/CLSI48h MIC, but not a CLSI24h MIC, of 0.25 mg/liter could be covered by targeting trough (upper 95% CI limit) levels of >2.4 mg/liter for the i.v./tablet formulation.

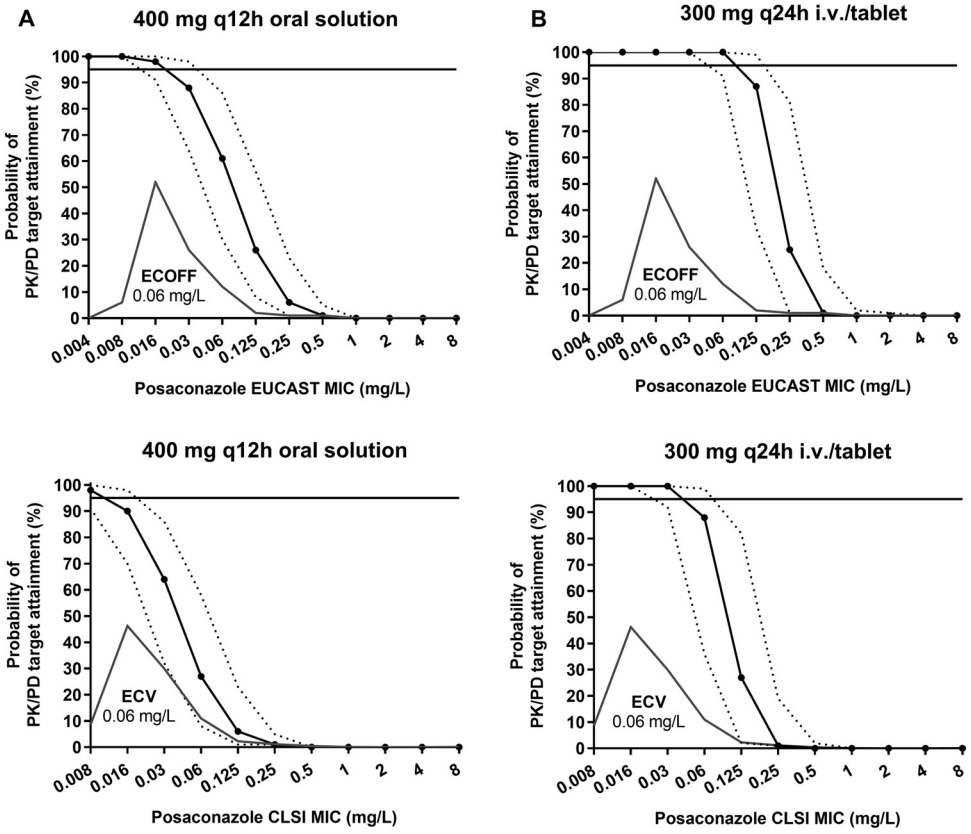

FIG 6 Target attainment rates for 5,000 patients receiving either (A) the oral solution of posaconazole (400 mg q12h) or (B) the newer formulations (300 mg i.v./tablet q24h), for which the AUCs were simulated with Monte Carlo for different EUCAST (top) and CLSI24h MICs (bottom). Horizontal lines correspond to 95% probability of target attainment (PTA). The i.v. and tablet formulation result in similar AUC results and therefore are presented together. Dotted lines correspond to 95% confidence interval of mean probability of target attainment (solid black line).

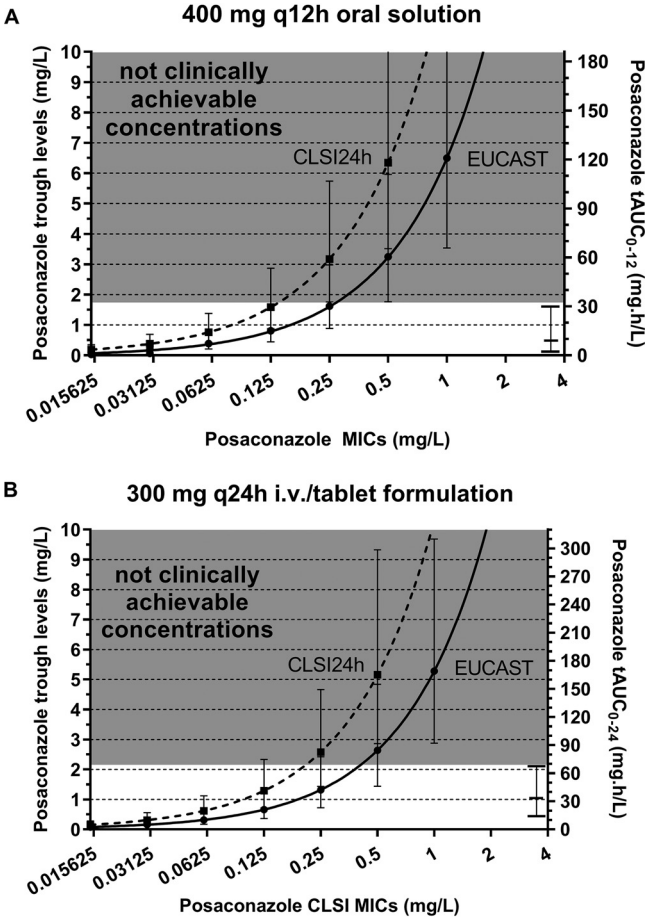

FIG 7 Target values for therapeutic drug monitoring of posaconazole for 400 mg q12h oral solution (A) and 300 mg q24h i.v./tablet (B) formulation. The trough levels and $tAUC_{0-24}$ obtained with each regimen are shown on the left lower part of the graphs. Error bars represent the 95% confidence interval.

Killing of *C. albicans* isolates was observed in the present study only at concentrations of 5 and 7 mg/liter (>20× MIC), as found previously in time-kill assays where no fungicidal activity was found at concentrations of ≤2 mg/liter (12). The *in vitro* pharmacodynamic range of effects are in line with previous animal studies in which kidney fungal burden increased by 3.29 ± 0.53 $\log_{10}$CFU/kidney from an initial 3.57 ± 0.37 $\log_{10}$CFU/kidney at the start of therapy, with a maximal effect up to 0 to 1 log killing (9). In the latter study, the $f\text{AUC}_{0-24}$/MIC was associated with a half-maximal effect (~2 log increase of initial fungal burden) of 6.12 to 26.7 using CLSI48h MICs. This target is lower than the *in vitro* 48-h PK/PD target of 169 (92 to 310) associated with half-maximal effect, which, however, corresponds to a 0.5 log increase, as opposed to the 2 log increase shown in animal PK/PD data. It is of note that the static PK/PD target in animals was around 100 to 200, which is close to the *in vitro* 48-h PK/PD EI_{50} target of 169 (92 to 310). Furthermore, previous animal survival studies with immunocompetent mice have shown 100% survival after 4 days of treatment with 1 mg/kg q24h of posaconazole against a wild-type isolate (MIC, 0.03 mg/liter) and with 25 mg/kg q24h against non-wild-type isolates with an MIC of 0.125 mg/liter but not with an MIC of 0.25 to 1 mg/liter (13).

Based on the PK/PD targets determined in preclinical models (100 $f\text{AUC}_{0-24}$/MIC), the *f*AUC values observed in human serum based on a protein binding of 98% (<0.6 mg · h/liter) can hardly cover the lower end of the wild-type population of *Candida albicans* isolates (MIC ≤ 0.06 mg/liter), despite the proven clinical response of posaconazole as prophylaxis against invasive candidiasis in hematological patients (14)

and as targeted therapy against esophageal candidiasis—with the caveat, however, that a local effect of the oral solution may contribute to the efficacy in the latter (1). *In vitro* comparative pharmacodynamic studies in the presence of serum showed that serum does not affect posaconazole's static exposure. Thus, protein-bound posaconazole is pharmacodynamically equally active compared to the unbound fraction. This could be explained in part because the protein-bound drug serves as a reservoir from which drug can be released when the free concentration drops. Another intriguing explanation may be the high affinity of posaconazole for the fungal cell membrane (15). Considering that cell-associated posaconazole levels were 50-fold higher than extracellular levels, and the 1 to 2% unbound fraction in human serum, posaconazole concentration in fungal membranes and thus at the site of action (sterol 14-demythalase in the inner face of the endoplasmic reticulum) could be 100% of the unbound fraction and thus equal to the total drug in human serum. Only when equating *f*AUC/MIC to *t*AUC/MIC can one estimate clinically relevant PTA, as done in the present study.

Although when protein binding is taken into account *in vivo*, azole EI_{50} PK/PD indices are harmonized, EI_{50} values correspond to relative effects (50% of maximum-minimum effect) rather than absolute effects (e.g., stasis, 1 log killing) that were commonly used for antibacterial drugs (16). The clinical significance of the EI_{50} endpoint is unknown. For fluconazole and voriconazole, the EI_{50} of ~25 *f*AUC/MIC in animal models corresponds to a 1 to 1.5 $\log_{10}$ CFU/ml decrease from drug-free control and a 1 to 1.5 log increase from initial inoculum, since the pharmacodynamic effects ranged from a 1 to 3 log increase from initial fungal burden (no stasis or killing was observed) (17, 18). However, based on clinical data for fluconazole, an *f*AUC/MIC of 100 is the clinically relevant PK/PD target (19) that corresponds to a near-maximal/near-stasis activity in animal models associated with a 2-log decrease from drug-free control or a 0.5 to 1 $\log_{10}$ CFU/kidney increase from initial inoculum (17). We have recently shown that an *f*AUC/MIC of 100 is also clinically relevant for voriconazole, which in *in vitro* models correspond to the EI_{50} (a 1.5 log decrease from drug-free control and a 2.5-log increase from initial inoculum) (11). For posaconazole, the pharmacodynamic effects ranged from a −1 to 3 $\log_{10}$ change from initial fungal burden, since killing was observed contrary to voriconazole and fluconazole where no killing was observed. After equating *f*AUC/MIC to *t*AUC/MIC of posaconazole, a *t*AUC/MIC of 100 in animal models was associated with a near-maximal/near-stasis activity (a 0.5 to 1 log increase from initial inoculum and a 3 log decrease from untreated animals) (9), whereas the *in vitro f*AUC/MIC of 100 in the absence of human serum corresponds to a half-maximal activity (EI_{50}) (a 2 log decrease from drug-free control and an ~1 log increase from initial inoculum), as with fluconazole and voriconazole. The difference between the near-maximal/near-stasis target in animals (~2 log decrease from drug-free control/~1 log increase from initial inoculum) and half-maximal activity target in serum-free *in vitro* studies that simulate serum concentrations may also be due to the presence of prolonged *in vivo* postantifungal effect compared to the absence of an *in vitro* postantifungal effect of azoles (9, 17, 18) or to differences between serum and kidney concentrations, as *in vivo* PKPD targets were assessed based on fungal burden in kidneys. Thus, we propose the near-stasis *in vivo* endpoint (2 log decrease from drug-free control/1 log increase form initial inoculum) and *in vitro* serum-free EI_{50} endpoint for analyzing pharmacodynamic effect for azoles. The near-stasis endpoint in neutropenic animal and *in vitro* studies may provide a clinical stasis with the help of neutrophils that usually exist in nonneutropenic patients with invasive candidiasis. Indeed, *in vivo* studies in neutropenic and nonneutropenic mice showed that median survival was prolonged and fungal load in the kidneys decreased by 1 $\log_{10}$ CFU in nonneutropenic mice compared to neutropenic mice (20).

Based on 48-h PK/PD targets of 330 and 169 $fAUC_{0-24}$/MIC found in the *in vitro* model for CLSI24h and EUCAST/CLSI48h methodologies, respectively, the oral solution would not cover the wild-type population (MIC ≤ 0.06 mg/liter) with high confidence, since the lower limit of PTA at the epidemiological cutoff (ECV/ECOFF) (0.06 mg/liter)

TABLE 2 Potential role of posaconazole against *Candida albicans* infections

Type of infection	*C. albicans* isolate EUCAST classification	Posaconazole treatment	Comment
Noninvasive	Wild type (MIC, ≤0.06 mg/liter)	Oral	TDM (C_{min} > 0.7 mg/liter)[a]
	Non-wild type (low-level resistance MIC, 0.125 to 0.25 mg/liter)	Oral with or without i.v./tablet	TDM (C_{min} > 2.4 mg/liter)[b]
	Non-wild type (MIC, >0.25 mg/liter)	None	
Invasive	Wild type (MIC, ≤0.06 mg/liter)	i.v./tablet	TDM (C_{min} > 0.7 mg/liter)[a,b]
	Non-wild type (MIC, >0.06 mg/liter)	None	

[a]C_{min} > 1.4 mg/liter for CLSI24h method.
[b]Consider combination therapy with an echinocandin in order to cover until the steady state is reached.

was 10 to 30%. In contrast, the i.v./tablet formulations would cover the entire wild-type population with much higher confidence, particularly that for EUCAST/CLSI48h (PTA > 95%) and less so that for CLSI 24h (PTA > 86%). Oral solution efficacy could be optimized by TDM targeting mean (95% CI) trough levels of >0.4 (0.21 to 0.7) mg/liter for EUCAST/CLSI48h and >0.76 (0.42 to 1.4) mg/liter for CLSI24h to cover the wild-type population. This in agreement with previous prophylaxis studies with oral solution in which trough levels of >0.3 to 1 mg/liter were associated with efficacy (21). The difference in target trough levels between CLSI24h and EUCAST/CLSI48h is because the EUCAST/CLSI48h MICs were one 2-fold higher than the CLSI24h MICs, as also shown in previous large comparative studies between CLSI24h and EUCAST methodologies for *C. albicans* and posaconazole MICs (22). The PTA was statistically significantly >95% for wild-type isolates with the CLSI48h but not with the CLSI24h method. Non-wild-type isolates with MICs of 0.125 and 0.25 mg/liter would require mean (95% CI) trough levels of >0.7 (0.4 to 1.2) and >1.3 (0.7 to 2.4) mg/liter for EUCAST/CLSI48h and >1.3 (0.7 to 2.4) and 2.6 (1.4 to 4.6) for CLSI24h with the i.v./tablet formulation, respectively, which are difficult to attain with the oral solution (>0.8 [0.4 to 1.5] and >1.6 [0.9 to 3] for EUCAST/CLSI48h and >1.6 [0.9 to 2.9] and 3.2 [1.8 to 5.7] for CLSI24h). Non-wild-type isolates with EUCAST/CLSI48h MICs of >0.25 mg/liter would require stable trough levels of >3 mg/liter that are difficult to obtain and may be associated with toxicity. An upper boundary of 3.75 mg/liter was suggested by the European Medicines Agency for average posaconazole plasma concentrations (23). Increasing the dose of the intravenous formulation from 200 to 300 mg resulted in an increase in adverse events (diarrhea, mucosal inflammation, headache, and rash) from 14% to 33% (8).

Another important point when treating invasive infections is when the steady state is reached which for posaconazole is after 5 to 7 days of therapy. This would be detrimental for treating invasive infections, given that mortality increases every day if targeted therapy is delayed (24). The AUC_{0-24} values on day 1 with a loading dose of 300 mg q12h of i.v. or tablet formulations were 1/2 and 1/3 of those on days 8 to 14, respectively (7, 8). Thus, targeting the necessary steady-state drug levels early will require 2 to 3× higher loading doses (600 mg q12h i.v. and 900 mg q12h for tablet formulation) on day 1 providing that these high loading doses result in high drug exposures and early TDM on day 2 for dose adjustment (25). Alternatively, bridging with posaconazole-echinocandin combination therapy until the target level is achieved would be necessary.

In conclusion, given that (i) posaconazole and fluconazole are equally active against oropharyngeal candidiasis (26), (ii) posaconazole retained activity against *Candida* isolates not susceptible to fluconazole (27) and also provides mold coverage relevant in some patient settings with invasive candidiasis, and (iii) fluconazole is efficacious and registered for invasive candidiasis, the potential role of posaconazole treatment against wild-type *Candida albicans* invasive infections and noninvasive infections by low-level-resistant *C. albicans* isolates might warrant further exploration in clinical studies (Table 2). For the treatment of invasive candidiasis with wild-type isolates, the i.v./tablet formulations of posaconazole may be an option since their exposures sufficiently cover the wild-type population. Oral solution could be used when i.v./tablet formulations cannot be given (due to intolerance to cyclodextrin or inability to swallow), provided that trough

(upper 95% CI limit) levels are >0.7 mg/liter for EUCAST (1.4 mg/liter for CLSI24h). Isolates with MICs of >0.06 mg/liter should be considered resistant. This PK/PD breakpoint is consistent with the current EUCAST susceptibility breakpoint of posaconazole against *C. albicans*. Higher loading doses of i.v./tablet formulation would be required to reach a steady state sooner, although this is off label and therefore needs to be tested clinically in dose escalation studies. TDM should be used to ensure sufficient exposure whenever treating invasive infections. This is because some patients will still have suboptimal levels without dose optimization. If delay in reaching a steady state is expected, combination therapy with an echinocandin may help posaconazole to attain PK/PD targets during non-steady state, since additive/synergistic interactions may lower the required target serum levels. Echinocandin therapy could be discontinued after 1 week of posaconazole therapy or when serial TDM indicates that the steady state is reached and trough levels are above the target levels. The role of posaconazole in treatment of invasive wild-type *Candida albicans* infections should be explored, particularly to provide an option in echinocandin-resistant cases for oral consolidation when mold prophylaxis is also warranted.

Finally, for noninvasive non-wild-type *Candida albicans* infections, TDM could be used to increase efficacy of posaconazole against low-level resistant isolates with EUCAST/CLSI48h MICs of 0.125 and 0.25 mg/liter, provided that trough (upper 95% CI limits) levels are >1.5 and >3 for the oral and >1.2 and >2.4 for the i.v./tablet, respectively (Table 2). However, since non-wild-type MIC distributions may also follow a normal distribution similar to the wild-type MIC distribution and one non-wild-type isolate with a MIC of 0.125 mg/liter could in fact have an MIC of 0.5 mg/liter, one should use the upper limit of non-wild-type MIC distribution (ECV/ECOFF of non-wild-type population) for TDM. If the non-wild-type ECV/ECOFF is 0.5 mg/liter for EUCAST/CLSI48h, posaconazole therapy could be optimized by TDM targeting trough (upper 95% CI limit) levels of >4.8 mg/liter, although this could be challenging in clinical practice.

MATERIALS AND METHODS

***Candida* isolates.** Three *C. albicans* strains (2-76, K1, and 98-210), previously tested in an animal model of disseminated candidiasis and with differential susceptibility profiles to posaconazole, were studied (9). Posaconazole MICs were determined in triplicate using the CLSI M27-A3 (28) and EUCAST methods (29) after 24 h. For the CLSI methodology, MICs were also determined after 48 h of incubation, as those MICs were used in original animal PK/PD experiments (9). The isolates were stored in normal sterile saline with 10% glycerol at −70°C and revived by subculturing on Sabouraud dextrose agar (SDA) plates supplemented with gentamicin and chloramphenicol (SGC2; bioMérieux). Inoculum suspensions were prepared in normal sterile saline from 24-h cultures and adjusted using a Neubauer counting chamber to a final inoculum of 10^4 CFU/ml, which was confirmed by subsequent culturing on SDA plates.

Antifungal drugs and medium. Pure powder of posaconazole (Merck Sharp & Dohme Corporation, Greece) was dissolved in sterile dimethyl sulfoxide (DMSO; Carlo Erba Reactifs-SDS, Val de Reuil, France), and stock solutions of 3.2 mg/ml were stored at −70°C until use. The medium used in the *in vitro* model was RPMI 1640 medium (with L-glutamine and without bicarbonate) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS; AppliChem GmbH, Darmstadt, Germany) and supplemented with 100 mg/liter chloramphenicol (AppliChem GmbH).

***In vitro* PK/PD model.** A modification of a previously described PK/PD dilution model was used (30). The model consists of a 100-ml culture vessel (conical glass flask) containing fresh RPMI 1640 medium at an initial volume of 30 ml for each *C. albicans* isolate and posaconazole dosing regimen simulated. The culture vessel is connected to a peristaltic pump (Minipuls Evolution; Gilson, Inc.), adding fresh medium in order to dilute its content at a rate comparable to the clearance of posaconazole in plasma in humans. Preliminary experiments using dialysis tubes in order to avoid dilution of fungi were unsuccessful in simulating posaconazole pharmacokinetics because of posaconazole binding on dialysis tubes's cellulose membrane. We therefore used a one-compartment model in which the volume of internal compartment increased over time, reaching ~120 ml at 48 h. Finally, the results of the model were compared with previous animal PK/PD data in order to validate the model.

***In vitro* pharmacokinetics.** Posaconazole levels were measured using a microbiological agar diffusion assay using the wild-type azole-susceptible isolate *Aspergillus fumigatus* AZN8196 (CLSI MIC, 0.03 mg/liter). Preliminary experiments showed that posaconazole was not stable in RPMI medium after freeze-thawing (25 to 50% loss at higher concentrations of frozen samples compared to fresh samples), while the addition of serum increased stability after thawing. In that context, 75 μl of each sample was collected from the model at each time point in cryovials (T311-2 Cryovial; Simport, Canada) and spiked with 25 μl heat-inactivated serum and stored at −70°C. Standard curve samples were also processed in the same way. Drug concentrations correlated linearly with the inhibition zone diameters ($R^2 > 0.98$) with an analytical sensitivity of 0.25 mg/liter and intraday/interday variation of <15%. A concentration-

time curve was generated for each simulated dose and isolate of the *in vitro* PK/PD model and analyzed by nonlinear regression analysis using a one-compartment model described by the equation $C_t = C_0 e^{-k/t}$, where C_t (dependent variable) is the concentration of drug at a given time t (independent variable), C_0 is the initial concentration of the drug at time 0 ($t = 0$ h), e is the physical constant 2.718, and k is the rate of drug removal. The half-life was calculated using the equation $t_{1/2} = k/0.693$ and compared with the respective values observed in mice. Finally, the area under the 24-h free drug concentration curve (fAUC$_{0-24}$) was calculated for each simulated dosage by applying the trapezoidal rule.

***In vitro* pharmacodynamics.** To estimate the fungal load inside the culture vessel of each posaconazole dosing regimen, 200-μl samples were collected at regular intervals up to 48 h, 10-fold serially diluted in normal saline, and subcultured on Sabouraud dextrose plates. Plates were incubated at 30°C for 24 h, and colonies were counted at each dilution. Dilutions that yielded 10 to 50 colonies were used in order to determine the $\log_{10}$ CFU/ml at each time point. Time-kill curves were constructed by plotting $\log_{10}$ CFU/ml over time.

Posaconazole pharmacodynamics in human serum. Given the high degree of protein binding of posaconazole (≥98%), preliminary experiments in the presence of 100% human serum were conducted to assess the impact of serum on posaconazole pharmacodynamics against the isolate *C. albicans* 2-76. Standard static time-kill assays in 100% human serum and in RPMI medium were conducted for 48 h. Total concentration (tC_{max}) 7 mg/liter in human serum and the corresponding maximum concentration of free unbound fraction in RPMI medium (fC_{max}) of 0.15 mg/liter was tested together with the fC_{max} of 7 mg/liter in RPMI medium, the tC_{max} of 0.15 mg/liter in human serum and their respective drug-free controls. Human serum was pooled from healthy volunteers, and after heat inactivation at 56°C for 45 min, it was stored at 4°C and used within 10 days.

***In vitro* animal correlation.** The *in vitro* PK/PD model was validated using the three *C. albicans* isolates previously used in a neutropenic murine candidiasis model (9). Briefly, groups of mice were infected with the three strains used in the present study and treated with three different posaconazole doses for 2 days. The dosages of 20, 80, and 320 mg/kg q24h, which corresponded to maximum mouse plasma concentrations (fC_{max}) of 0.15, 0.25, and 0.5 mg/liter, respectively, and an average half-life of 15 h were simulated in the *in vitro* PK/PD model. Higher posaconazole exposure of fC_{max} values of 5 and 7 mg/liter were also evaluated in the *in vitro* model in order to better describe the exposure-effect relationship. Drug concentrations were added at the corresponding fC_{max} values in the *in vitro* model once daily for 2 days. The $\log_{10}$CFU/ml and posaconazole levels were determined at regular time intervals as described below. The EI_{50} values resulted from the *in vitro* exposure-response relationship (change in $\log_{10}$CFU/ml from $t = 0$ h versus fAUC$_{0-24}$/MIC) at 24 h and 48 h of incubation were analyzed with the E_{max} model as described below and compared with the EI_{50} of the *in vivo* exposure response curve after 2 days of treatment (change in $\log_{10}$CFU/kidneys versus fAUC$_{0-24}$/MIC) (9). The *in vivo* fAUC$_{0-24}$ was calculated on the basis of the 2% unbound fraction of posaconazole in animal plasma. Two independent experiments were conducted.

PK/PD analysis. The PK/PD index fAUC$_{0-24}$/MIC ratio was calculated for each simulated dose, isolate, and experiment. The drug exposure-response relationship, expressed with the 48-h $\log_{10}$CFU/ml reduction for each dosing regimen and isolate compared to the start of therapy values versus AUC$_{0-24}$/MIC, was analyzed with nonlinear regression analysis using the sigmoidal model with variable slope (E_{max} model) described by the equation $E = (E_{max} - E_{min}) \times EI^n/(EI^n + EI_{50}{}^n) + E_{min}$, where E_{max} is the maximum increase in $\log_{10}$CFU/ml in drug-free control (kept constant to $\log_{10}$CFU/ml in drug-free control), E_{min} is the minimum in $\log_{10}$CFU/ml found at high-drug exposures (kept constant to the maximal effect observed), EI is the exposure index fAUC$_{0-24}$/MIC, EI_{50} is the exposure index required to achieve 50% of E_{max}- E_{min}, and n is the slope of the dose-effect relationship (Hill coefficient). Because the volume of the *in vitro* PK/PD model increased over time, a similar analysis was performed using the actual $\log_{10}$CFU after multiplying the CFU/ml with the volume at each time point. The goodness of fit of the E_{max} model was assessed by visual inspection of graphs, residuals analysis, postrun tests, and R^2. All data were analyzed using the statistics software package Prism version 5.0 for Windows (GraphPad Software, San Diego, CA).

Prediction of PK/PD target attainment. Using the PK/PD targets derived from the PK/PD analysis, Monte Carlo simulation analysis was performed using the "Normal random number generator" function of Excel (Microsoft Office 2007) for 5,000 patients receiving the oral solution of posaconazole 400 mg q12h, which corresponds to a steady state mean ± SD AUC$_{0-12}$/AUC$_{0-24}$ (trough levels) of 8.62 ± 7.41/17.24 ± 14.82 mg · h/liter (0.723 ± 0.63 mg/liter) (31), or the new tablet and i.v. formulations of posaconazole 300 mg q24h, which correspond to steady-state mean ± SD AUC$_{0-24}$ (trough levels) of 35 ± 14.31 (average concentration of free unbound drug [C_{avg}], 1.46 ± 0.55 mg/liter) (7) and 34.3 ± 12.3 mg · h/liter (C_{avg}, 1.42 ± 0.60 mg/liter; minimum concentration of free unbound fraction [C_{min}], 1.07 ± 0.58 mg/liter), respectively (8). Since serum did not alter static exposure of posaconazole in the preliminary and previous posaconazole pharmacodynamics experiments (32), the fAUC$_{0-24}$/MIC is pharmacodynamically similar to tAUC$_{0-24}$/MIC. Thus, the proportion of patients attaining the *in vitro* EI_{50} AUC$_{0-24}$/MIC for the solution or the i.v./tablet dosing regimens was determined for different MICs ranging from 0.016 to 4 mg/liter for each of the two methodologies. Recently published MIC distribution data for *C. albicans* with CLSI (33) and EUCAST (34) were used.

Therapeutic drug monitoring. In order to determine the highest MIC that can be covered with three dosing regimens and the target drug exposures for therapeutic drug monitoring of posaconazole, AUC$_{0-24}$ values necessary to attain the EI_{50} target for q12h oral solution and q24h i.v./tablet dosing regimens were correlated with different EUCAST and CLSI MICs. The corresponding trough levels were

estimated based on steady-state AUC/trough ratios previously published for oral solution 400 mg q12h (AUC_{0-12}/trough = 13) (31) and 300 mg i.v./tablet q24h (AUC_{0-24}/trough = 32) (7, 8) dosing regimens.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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We have no conflicts to declare.

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